



0575/56614/JPW/AJM/CY

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant : Mercy M. Davidson

Serial No. : 09/604,876

Examiner: R. Schnizer

Filed : June 28, 2000

Group Art Unit: 1635

For : IMMORTALIZATION OF HUMAN POST-MITOTIC CELLS

1185 Avenue of the Americas
New York, New York 10036

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

DECLARATION UNDER 37 C.F.R. § 1.132

I, Mercy M. Davidson, Ph.D., hereby declare that:

1. I am the inventor named in the above-identified patent application.
2. I am also presently a Research Scientist in the Department of Neurology at Columbia University in New York, NY. A copy of my curriculum vitae is attached hereto as **EXHIBIT A**.
3. I have reviewed and am familiar with the subject application, including pending claims 1 and 3-5. I understand that pending claims 1 and 3-5 provide an immortalized human undifferentiated cardiomyocyte cell line wherein the cell line is produced by a method comprising the step of fusing a post-mitotic primary non-immortalized human cardiomyocyte with a human fibroblast, the fibroblast (a) having been treated with ethidium

*Considered
Done
7/31/00*

Applicant : Mercy M. Davidson
Serial No. : 09/604,876
Filed : June 28, 2000
Page 2

bromide; (b) comprising a replicable vector expressing SV40 large T antigen which confers immortality on a cell comprising same; and (c) being free of mitochondrial DNA.

4. I have read and am familiar with the January 27, 2004 Office Action issued by the United States Patent and Trademark Office in connection with the subject application. I understand that in the January 27, 2004 Office Action, the Examiner rejected claims 1 and 3-5 under 35 U.S.C. §102(b) as allegedly anticipated by Wang et al., "Establishment Of A Human Fetal Cardiac Myocyte Cell Line", In Vitro Cell. Dev. Biol., 27A:63-74, January 1991 (hereinafter "Wang"). A copy of Wang is annexed hereto as **EXHIBIT B**.
5. Specifically, I understand the rejection under 35 U.S.C. §102(b) to be based on the Examiner's assertion that the human fetal cardiac myocyte cell line taught by Wang, i.e., the "W1" cell line, is the same as the cell line of claims 1 and 3-5 (i.e., the "claimed cell line").
6. I have read and am familiar with Wang and its teachings, in particular that of the W1 cell line.
7. The claimed cell line is not the same as the W1 cell line of Wang for the reasons set forth below.
8. Images of the claimed cell line are shown in **EXHIBIT C**. Specifically, **EXHIBIT C** sets forth two

light microscopic images of cells from a transformed human adult cardiomyocyte cell line designated "AC16" (panel (a)) and a transformed human fetal cardiomyocyte cell line designated "RL14" (panel (b)). Both the AC16 and RL14 cell lines are immortalized human undifferentiated cardiomyocyte cell lines. The AC16 cells were generated by fusing post-mitotic primary non-immortalized human cardiomyocytes obtained from primary adult human ventricular tissue with ethidium bromide-treated, human SV-40-transformed mitochondrial DNA-free (rho zero) fibroblasts. The RL14 cells were generated by fusing post-mitotic primary non-immortalized human fetal cardiomyocytes with the same ethidium bromide-treated, human SV-40-transformed mitochondrial DNA-free (rho zero) fibroblasts used to generate the AC16 cells. The only difference between the AC16 and RL14 cell line production methods was the use of primary cardiomyocytes at developmentally different stages (i.e., adult or fetal). Adult stage primary cardiomyocytes were used to generate the AC16 cell line, and fetal stage primary cardiomyocytes were used to generate the RL14 cell line.

9. The claimed cell line, whether derived from adult or fetal primary cardiomyocytes, possesses certain morphological characteristics. As shown in **EXHIBIT C**, the AC16 and RL14 cell lines are characterized by homogeneous cells which (a) are evenly-shaped, (b) have large central nuclei and

(c) have a larger average ratio of nucleus volume to cytoplasm volume than do native primary human cardiomyocyte cells. Both AC16 cells and RL14 cells are also smaller in size relative to native primary human cardiomyocytes.

10. Figures 1 and 5 of Wang show the W1 cell line. In contrast to the claimed cell line, the W1 cell line shown in Figures 1 and 5 of Wang contains heterogeneous, refractile, spindle-shaped cells. The cells of the W1 cell line morphologically resemble native human primary cardiomyocytes. Wang states that the W1 cell line "has been shown to share morphologic and phenotypic characteristics with native human fetal cardiomyocytes," that W1 cells "look very similar" to native primary cardiomyocytes under light and electron microscopy, and that "by morphologic criteria these two types of cells [W1 cells and human fetal cardiac myocytes] are indistinguishable from one another." Wang, page 73, column 1, ¶1; page 66, column 1, 2nd full paragraph; and page 66, legend for Figure 1, respectively.

*Same
conditions*

11. A comparison of morphologies of the claimed cell line (described in paragraphs 8 and 9) and the W1 cell line of Wang (described in paragraph 10 above) reveals that these two cell lines are morphologically different, and therefore, not the same.

Applicant : Mercy M. Davidson
Serial No. : 09/604,876
Filed : June 28, 2000
Page 5

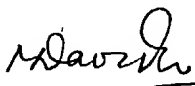
12. The claimed cell line and the W1 cell line of Wang also have different growth characteristics. **EXHIBIT D** sets forth growth curve data obtained from an experiment using AC16 cells. In this experiment, 5×10^4 cells of each of the three cell types (AC16, control fibroblasts and DWFb1p⁰ (human SV-40 transformed mitochondrial DNA-free [rho zero] fibroblasts)) were seeded in 10 ml of growth medium in multiple 10 mm² dishes. For AC16 cells, DMEM/F12 supplemented with 12.5% FBS was used, and for control fibroblasts, MEM supplemented with 15% FBS was used. To support the growth of DWFb1p⁰ cells, medium was used which consisted of the fibroblast medium and uridine at 50µg/ml. At 24-hour time intervals, cells from individual plates were trypsinized and counted. AC16 cells continued to divide until the 6th day. Proliferation of AC16 cells slowed as the cells approached confluence, similar to the observed proliferation of control primary fibroblasts. The calculated doubling time was 24.49 hours for the AC16 cells, 23.91 hours for the control fibroblasts and 23.09 hours for the DWFb1p⁰ cells.
13. Wang states on page 66, column 2, 1st full paragraph, that the W1 cell line has a doubling time of 55.4 hours. This doubling time is more than twice as long as the 24.49 hour doubling time of the claimed cell line.

Same conditions?

Applicant : Mercy M. Davidson
Serial No. : 09/604,876
Filed : June 28, 2000
Page 6

14. Based on the morphological and doubling time differences described above, the claimed cell line is not the same as the W1 cell line of Wang.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made herein on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the subject application or any patent issuing thereon.



Mercy M. Davidson, Ph.D.

5. 18. 04

Date